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Interpretation of Discordant Results in Maternal/Newborn Dyad Drug Screening

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Abstract: Background: Identification of *in utero* illicit drug exposure has paramount importance in medical care and well-being of the newborn. Newborn drug screening has traditionally been performed on meconium; however, umbilical cord tissue has gained popularity as an alternative specimen. We present six cases of newborn drug testing results from different specimens to highlight the potential inconsistencies and challenges with interpretation. Methods: Six infants born to mothers with illicit drug use who underwent drug screening are reviewed. Analysis was performed on meconium, umbilical cord tissue, newborn and/or maternal urine samples. Available meconium and umbilical cord tissue were analyzed using immunoassay and confirmed by HPLC-MS/MS. Urine drug screening was performed on available specimens using Enzyme-Multiple Immunoassay Technique and confirmed using HPLC-MS/MS. IRB approval for the study was granted by the University of Louisville and University of Louisville Hospital. Results: In each case presented there was significant variation in toxicology results between maternal/infant urine, meconium, and umbilical cord tissue analysis. Conclusions: Discrepancies in drug screening results from different specimens have been observed in the six mother/infant dyads presented. The utility of each specimen is dependent on several considerations and may warrant the testing of different sample types. Review of potential causation for conflicting results can help clinicians to select the proper tests and assist with interpretation when results deviate between the types of specimens analyzed.

Keywords: Newborn Drug Screening, Meconium, Umbilical Cord, Illicit Drug Use, Pregnancy

1. Introduction

An estimated 15% of neonates are affected by prenatal drug exposure each year, posing substantial health risk for the mother and baby [1]. Subjection to these substances is tough to measure as they can include indirect, prescribed, and illicit/recreational exposures. The significance involves the resultant wide spectrum of detrimental effects to the infant, including a collection of withdrawal symptoms referred to as neonatal abstinence syndrome (NAS) [3, 4] and increased risks for difficulties with future physical and cognitive development [5, 6]. The estimated national rate of NAS per 1,000 newborn hospitalizations is 6.8% (2018 Healthcare Cost & Utilization Project). The type and severity of the withdrawal symptoms depends on the drug type, duration of exposure, and how the body metabolizes the drug which are the same features critical in the ability to detect the substances abused. Identification of effective drug screening tools is essential for early diagnosis of drug exposure, treatment, accuracy of results and interpretation for legal custody resolutions.

Newborn drug screening is recommended for all neonates that present with clinical symptoms associated with prenatal drug exposure, maternal history of drug use and/or results of maternal drug testing [7, 8]. When drug testing is indicated, toxicology testing can be performed on specimens from the mother (urine) and the baby (urine, meconium, umbilical cord tissue, and/or rarely hair) [9]. Since a clear guidance is absent, hospital-specific practices prevail. The risk-based drug screening program at the University of Louisville Hospital encourages mothers to consent to urine drug testing. Any infant suspicious for intrauterine illicit drug exposure undergoes testing. As part of our institutional policy, an

initial drug screening is performed on maternal and first void newborn urine (if possible) based on maternal history and presentation. All presumptive positive urine results are to be sent to a reference laboratory for confirmation by HPLC-MS/MS, but confirmation may not be possible if there is an insufficient amount of urine collected. Meconium and/or umbilical cord tissue specimens are collected and then processed by an outside reference laboratory drug screening for HPLC-MS/MS confirmation.

The gold standard specimen in newborn drug screening has historically been meconium [31, 35] Meconium, the first feces of a newborn is a heterogeneous material of water, desquamated epithelial cells, fine surface hair (lanugo), fatty material from vernix, bile salts, cholesterol/sterol precursors, mucopolysaccharides, sugars, lipids, proteins, trace metals, various pancreatic/intestinal secretions, and residue from swallowed amniotic fluid [7]. Formation begins as early as the 12th week of gestation and has a detection window for drugs from the second trimester throughout the third trimester [7, 10] (Figure 1). As described by Gareri et al fetal swallowing, which appears by approximately 13 weeks gestation, is the primary method for drug concentration into meconium [10, 11]. Drugs deposit into meconium starting with maternal drug consumption followed by circulation of the drugs and their metabolites to the placenta and then to the fetal circulation. Factors that influence the efficiency of placental drug transfer include molecular size, ionization state, lipophilicity, and plasma protein/placenta tissue binding. Drugs most abused are of small molecular size, highly lipophilic and easily transferred from the placenta to the fetal circulation by passive diffusion. The fetus can excrete some drugs into the bile and then directly to the meconium but more commonly described are drugs/metabolites excreted in fetal urine and then into the amniotic fluid [10, 11]. Fetal swallowing of the amniotic fluid then concentrates the drugs/metabolites in the meconium, serving then as a "preserved record of fetal drug exposure". Meconium toxicology requires collection whenever it is passed, may require several days to collect enough for testing which may impact specimen/drug/metabolite stability, and can be missed entirely if discarded before it can be collected or passed into the amnionic fluid before delivery [7].

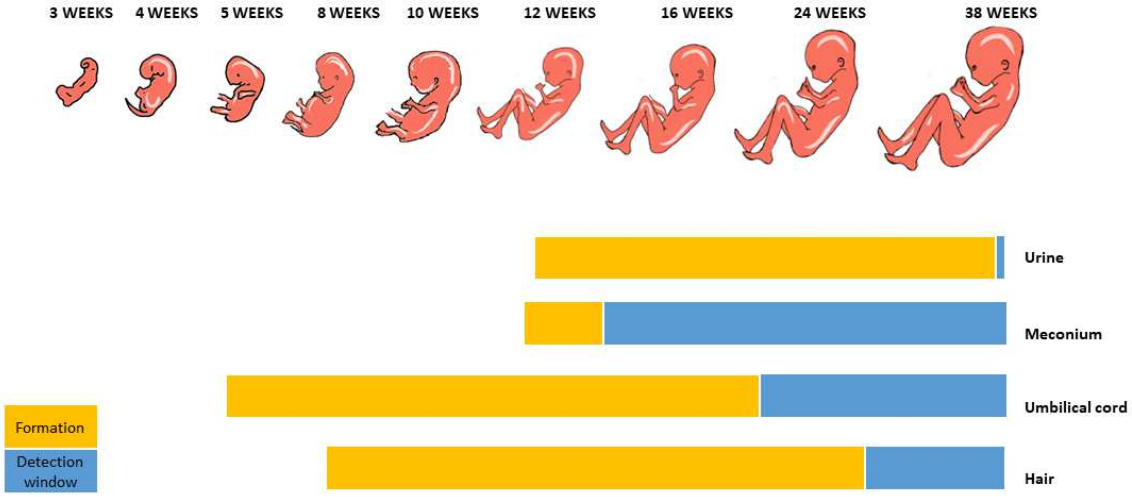

Figure 1. *Detection window periods for drugs detection in urine, meconium, umbilical cord, and hair specimens.*

Due to these challenges, umbilical cord tissue has been introduced as an alternative specimen for drug screening in neonates [12, 13, 14]. Umbilical Cord tissue is formed during the 7th week of gestation and has a drug/metabolite detection window of the last 20 weeks of gestation, reflecting drug exposure during the third trimester [15] (Figure 1). Unlike meconium, the mechanism of deposition of drugs in the umbilical cord tissue is not fully understood (7).

Previous studies have compared newborn drug testing results in paired umbilical cord and meconium samples involving prescribed opioids and found some agreement between the two sample types [2, 16, 17, 18, 30, 31]. Studies involving comparisons of specimens from subjects exposed to multiple illicit drugs however is scarce and conflicting. As for example, Colby et al indicated meconium was more sensitive than umbilical cord tissue for neonatal drug exposure with a concordance between the two matrices of 70%. Cotton et al reported umbilical cord tissue was more sensitive with shorter length in time to receive results [30]. Wabuyele et al in their review of detecting drug exposed newborns, indicated "because of variations in analytical methods, limited numbers of positive results identified in studies published thus far, limited understanding of comparable cutoffs required for detection, and limited recognition of best content, it remains to be seen whether umbilical cord tissue and meconium can be used interchangeably to detect drug-exposed newborns and particularly to identify those at high risk of NAS and other post-partum consequences" [16]. Such findings support there can be limitations in the ability to predict the concordance between umbilical cord and meconium in cases of utero illicit drug exposure.

We present a series of six clinical cases to illustrate some of the practical challenges of interpreting drug testing when urine, meconium and/or umbilical cord tissue are all sent from the same patient for toxicology analysis. Discussions of each Case will provide further understanding of specimen limitations and assist clinicians/laboratorians with the interpretation of toxicology specimen results during the evaluation of prenatal drug exposure.

2. Methods

Retrospective review of six infants born to mothers with illicit drug use who underwent newborn drug screening. Analysis was performed on meconium, umbilical cord tissue and urine samples. Available meconium and umbilical cord tissue were analyzed using immunoassay and confirmed by HPLC-MS/MS. Urine drug screening was performed on available specimens using Enzyme-Multiple Immunoassay Technique and confirmed using HPLC-MS/MS. Local IRB approval for the study was granted by the University of Louisville and University of Louisville Hospital.

3. Case Results

Table 1 shows the results of the six maternal/newborn dyads who underwent toxicology testing.

Table 1. *Drug testing results from maternal and/or newborn urine, umbilical cord, and meconium samples.*

	Detected Substances	Maternal Urine	Newborn Urine	Umbilical Cord	Meconium
CASE I	Amphetamine	+	+	+	+
	Methamphetamine	+	+	+	+
	Morphine	ND	ND	+	+
	Codeine	ND	ND	ND	+
	Benzoylecgonine	ND	ND	+	+
	m-OH-BZE	ND	ND	ND	+
CASE II	Amphetamine	+	NA	+	+
	Methamphetamine	+	NA	+	+
	Buprenorphine	+	NA	ND	+
	Norbuprenorphine	ND	NA	ND	+
CASE III	Methadone	+	ND	+	+
	EDDP	ND	ND	+	+
	Carboxy THC	+	ND	+	ND
	Amphetamine	+	ND	ND	ND
CASE IV	Cocaine	ND	NA	+	+
	Benzoylecgonine	ND	NA	+	+
	m-OH-BZE	ND	NA	ND	+
	Cocaethylene	ND	NA	ND	+
	Amphetamine	+	NA	ND	ND
CASE V	Barbiturates	+	ND	NA	ND
	Opiates	+	ND	NA	ND
	Morphine	+	ND	NA	ND
	Cocaine	+	+	NA	+
	Benzoylecgonine	+	+	NA	+
	m-OH-BZE	ND	ND	NA	+
	Cocaethylene	ND	ND	NA	+
CASE VI	Fentanyl	ND	NA	+	ND
	Norfentanyl	ND	NA	+	ND
	Buprenorphine	+	NA	ND	ND
	Benzodiazepines	+	NA	ND	ND
	Cocaine	+	NA	ND	ND
	Benzoylecgonine	ND	NA	+	ND

ND=Compound not detected. NA=Sample not available.

4. Discussion of Cases

4.1. *CASE I*

In Case I, Methamphetamines (MAMP) and Amphetamines (AMP) were present in all four specimens (maternal/newborn urine, umbilical cord, meconium). The interpretation of MAMP toxicology is complex as it can be present in prescribed/over the counter medications, or it can be a substance of abuse characterized as a highly addictive central nervous system (CNS) stimulant with potential life-threatening effects. A single dose of MAMP can be detected in urine up to 5–16 hours, however, the half-life may increase up to 30 hours when urine is alkalized. MAMP is eliminated in the urine in two forms, (d) dextrorotary (potent CNS stimulant and responsible for abuse) and/or (*l*) levorotary enantiomer (low CNS activity and abuse potential, i.e. Vicks Vapor Inhaler). Chiral analysis as opposed to mass spectral testing is required to distinguish the subtle structural differences between the two enantiomers [34]. In this Case both the newborn and maternal urine toxicology were positive for AMP/MAMP indicating a more acute exposure while the presence of both compounds in meconium and the

umbilical cord tissue would indicate a prolonged exposure during the pregnancy. These findings are also supported by the positive result of AMP in maternal urine screening a few months prior to delivery and admittance of the mother to methamphetamine abuse.

Toxicology results from this Case also detected morphine in meconium and the umbilical cord tissue while Codeine was present only in meconium. Neither morphine nor codeine were present in the analyzed maternal/newborn urine. These results are indicative of a prior but not recent opioid exposure. Morphine is a natural alkaloid from 'Poppies", and its detection can reflect use of morphine, codeine, heroin, and the ingestion of poppy seeds. Poppy seeds are derived from the pods of papaver somniferum (poppy plant) and can be contaminated with the plant's opium milk, which contains the morphine. The seeds are usually cleansed/processed before use as an ingredient but may still contain opiate residue. The contamination is not of high enough concentration for morphine-like effects but can be enough to cause a positive result on a sensitive toxicology test and should be considered when morphine is identified on any drug screen. Codeine, as detected on meconium toxicology in this Case, is a semisynthetic opioid synthesized by methylation of morphine, which makes codeine less polar compared to morphine [23]. When administered, Codeine undergoes O-dealkylation by the drug-metabolizing enzyme CYP2D6 resulting in the release of morphine and other metabolites, to give Codeine the characteristically known effects of morphine.

To explain discordance between meconium and umbilical cord testing in this Case, it is known there are key differences in their physicochemical matrices. Meconium has a much more lipophilic matrix compared to umbilical cord tissue and may explain why codeine being less polar was not found in umbilical cord tissue but was present in meconium. J. Colby et al have alluded to drug deposition based on lipophilic drug characteristics and found umbilical cord tissue to be more often positive for amphetamines, barbiturates, and benzodiazepines, while meconium more often positive for buprenorphine, cannabinoids, cocaine, methadone, and other opioids (30). Likewise, Pandya et al also explain discordance between meconium and umbilical cord tissue as the result of hydrophilic characteristics of the matrices and deposition of various drugs/metabolites [35].

Since codeine and morphine were not present in the maternal urine suggest an opioid exposure early in pregnancy and abstinence later in gestation which was confirmed by the patient's medical history.

Also, in this Case Benzoylecgonine, a cocaine metabolite, was detected in meconium and the umbilical cord tissue while m-OH-BZE, a minor cocaine metabolite, was present in the meconium alone. Neither were detected in urine specimens. Detection of cocaine metabolites in the urine is limited due to the relatively short detection window (up to 3 days) and variability in elimination of cocaine and its metabolites. Although cocaine has a short half-life (<1 h), half-lives of its metabolites are substantially longer (6-8 hours) [24]. In this case results would suggest a use of cocaine earlier in gestation with recent abstinence or consumption/exposure below limits of detection. In general, a negative drug result despite recent or ongoing use can be found in four different conditions: the window of detection of the specific drug has passed, the substance of abuse cannot be detected by the standard testing panel, the drug concentration in the sample is below the detection limits of the analysis or in cases of an adulterated sample.

4.2. *CASE II*

In Case II, AMP and MAMP were detected in meconium and umbilical cord tissue with a positive result in the maternal urine drug screen, suggesting both recent and prolonged use of MAMP during the pregnancy. Urine from infant was not available for testing. A documented history of maternal drug abuse of MAMP supports these findings.

Buprenorphine and its predominant metabolite, norbuprenorphine, were also detected in meconium while buprenorphine but not norbuprenorphine was found in the maternal urine drug screen.

Both meconium and maternal urine toxicology results suggest buprenorphine use early in gestation and more recent drug exposure. Buprenorphine is a semi-synthetic opioid derived from thebaine with a highly lipid soluble base. Its half-life is 24 – 42 hours, and is metabolized rapidly in the liver, excreted into the urine/feces, and may explain why the metabolite norbuprenorphine was not yet detected in the urine.

Neither buprenorphine nor norbuprenorphine were present in umbilical cord tissue. Since meconium is more highly lipophilic compared to umbilical cord tissue may explain why the highly lipophilic buprenorphine and norbuprenorphine were more readily deposited into meconium compared to umbilical cord tissue.

4.3. *CASE III*

In Case III, maternal urine, umbilical cord tissue and meconium were all positive for methadone. On the contrary, methadone was negative in the neonatal urine sample. Methadone is a synthetic opioid, a non-polar molecule and can cross the placental barrier freely resulting in sometimes unpredictable detection/effects. Methadone and its metabolites are present in urine up to 5 days, and the half-life of methadone ranges from 15 to 60 hours. A false negative neonatal urine toxicology result can be attributed to an early methadone use during pregnancy, a urinary drug concentration below the cutoff for positivity, sample dilution of the urine or delayed collection [25, 26]. In this case the negative urine result was felt to be a combination of delayed specimen collection (i.e. not the first void) and perhaps drug concentration below detection. Methadone is primarily eliminated via hepatic metabolism by enzymatic CYP 450 demethylation and cyclization to produce several inactive metabolites, primarily EDDP and 2-Ethyl-5-methyl-3,3-diphenyl-1-pyrroline (EMDP). Studies have found that variation in CYP 450 polymorphism can result in a

significant decrease in the metabolic ratio of methadone and in this case may explain the negative maternal/infant urine drug screen for EDDP despite its presence in both meconium and umbilical cord tissue [27].

The presence of carboxy THC in the maternal urine indicates recent cannabinoid exposure. Negative meconium sampling but positive umbilical cord tissue for carboxy THC is more challenging to explain. J. Colby et al have indicated drug deposition based on lipophilic drug characteristics and implied cord tissue to be less often positive for cannabinoids than meconium [30]. Carboxy THC present in umbilical cord tissue and not meconium may be related to a concentration below a reportable detection limit for meconium but of a sufficient reportable detection level in umbilical cord tissue. Umbilical cord drug concentrations are typically much lower than in meconium, and as such have lower analytical limits of detection compared to those of meconium [16]. Another possible but unlikely explanation could be contamination/improper collection of the cord tissue. Carboxy THC containing blood residue from an acute exposure that was not properly flushed during cord collection may have contaminated the specimen resulting in an inaccurate interpretation there was earlier carboxy THC exposure [16].

In this case the maternal urine drug screen was also positive for AMP/MAMP, but was negative in umbilical cord tissue, meconium, and newborn urine drug screen. Maternal urine drug screening in this case was not confirmed with HPLC/MS-MS analysis, therefore a false result cannot be ruled out since immunoassay specificity for AMP/MAMP is low resulting in a higher incidence of false positive results [28, 29]. As alluded earlier in Case I, urine screenings for AMP/MAMP commonly involve the use of immunoassays which often lack the specificity to target individual drugs and typically screen for structurally related compounds. Immunoassays are utilized since they provide rapid results, cost relatively little, and are commercially available. False positives for AMP/MAMP are possible and must be considered when interpreting results and should always be confirmed with gas chromatography-mass spectrometry testing and if indicated chiral analysis.

4.4. CASE IV

In Case IV maternal urine drug screen was negative for cocaine, whereas meconium and umbilical cord specimens were positive for cocaine and its metabolites. Cocaine's half-life is <1 h, so it can be detected in urine for up to one day after last using the drug. Benzoylecgonine, a cocaine metabolite, has a longer half-life of 6-8 hours and can be detected in urine up to 3 days after last exposure. As for this Case, the data suggest an abstinence from cocaine at least up to ~ 3 to 4 days prior to delivery. The absence of cocaine in the maternal urine may reflect the detection window of the drug and its metabolites has passed (i.e. 4 days) but could be related to testing interference, sample deterioration, and concentrations below the reporting limits of positivity in the screening method. Cocaethylene is the ethyl ester of benzoylecgonine. When cocaine and ethanol are consumed together, cocaine is metabolized in the liver to cocaethylene. Like cocaine, this metabolite blocks the reuptake of dopamine at the synaptic cleft and its plasma half-life is three to five times that of cocaine. The result potentiates the toxic effect of cocaine with an increase in myocardial oxygen demand and a higher risk for mortality [32]. If cocaethylene is identified on drug screening, there should also be a concern for alcohol use/abuse and potentially more significant side effects. Cocaine as a dopamine and norepinephrine reuptake inhibitor results in their accumulation at the synaptic cleft which can then cause tachycardia, hypertension, arrhythmias, and vasoconstriction. The vasoconstriction effect in pregnancy can compromise uteroplacental blood flow and conceivably directly affect the fetus. In this Case the infant presented with congenital malformations and was small for gestational age, but it is unknown if these findings were a result of intrauterine cocaine exposure.

Also, in this Case AMP was found only present in maternal urine, suggesting a recent exposure to AMP however as previously discussed the interpretation of MAMP/AMP toxicology is complex with a low immunoassay specificity and high false positive rate. Any toxicology results for MAMP/AMP should be confirmed with gas chromatography-mass spectrometry testing and if indicated chiral analysis [28, 29].

4.5. ***CASE V***

In Case V, the maternal/neonatal urine and meconium analysis were positive for cocaine and its metabolites. Agreement of the results suggest a recent exposure and possible long-term use of cocaine. As previously noted, cocaine has a short half-life and can be detected in urine up to one day, while cocaine metabolites can be detected in urine up to 3 days. As in Case IV, the detection of the cocaine metabolite, cocaethylene should also raise the concern for alcohol use/abuse and hypothetically a greater risk for adverse potent vasoconstrictive effects on placental circulation.

Maternal urine was also positive for barbiturates and opioids, while meconium and neonatal urine were negative for these analytes. The mother's urine was collected on admission prior to any hospital administered medications. The inconsistency in results may be associated with the timing of the last dose (i.e. acute drug exposure) and insufficient time for drug accumulation. A possible delay in collecting the neonatal urine specimen may also lead to false-negative results and explain, as in this case, the negative urine toxicology.

4.6. ***CASE VI***

In Case VI, newborn urine drug screening was not performed. Maternal urine toxicology obtained was positive for buprenorphine, benzodiazepines, and cocaine which would indicate at least recent use of these drugs. Discussion

is given in Case II with regards to Buprenorphine and simulates for Case VI. Of note Benzodiazepines found on urine drug screens are limited in identifying specific benzodiazepines and there are known false positives with various drugs such as Sertraline.

Benzoylecgonine (i.e. Cocaine metabolite) was present in umbilical cord tissue but absent in meconium and maternal urine. Pandya et al indicated they found the positivity rate for cocaine and cocaine metabolites to be higher in meconium than umbilical cord tissue, however Benzoylecgonine was noted to be the most frequent cocaine metabolite detected in umbilical cord tissue [35]. In this Case the Benzoylecgonine found in umbilical cord tissue would suggest cocaine was used sometime in the later period of the pregnancy with the possibility of earlier exposure but inadequate detectable levels in meconium. Cocaine found in the urine but not Benzoylecgonine, would support an acute exposure without time for metabolite production to reach detectable levels in the urine. Since drug concentrations in umbilical cord tissue are generally lower compared to meconium, HPLC-MS/MS assays by design have lower umbilical cord tissue positivity cut-off concentrations compared to meconium. In this Case, benzoylecgonine may be present in meconium but not of a concentration to meet the cut-off to be recorded as positive whereas umbilical cord levels despite being lower are high enough to meet the positivity cut-off concentration.

In this Case also identified were fentanyl and norfentanyl in umbilical cord tissue but not present in meconium and likewise would indicate use in the later period of pregnancy but not recent enough to be detected in the maternal urine specimen. In this Case there was no maternal documentation of prescribed use of fentanyl, including a KASPER review. Because of its lethal potency, detection of Fentanyl as a substance of abuse should be of extreme concern. Fentanyl, classified as a μ-opioid receptor agonist (MOP, IUPHAR classification), originally was derived from the synthetic opioid meperidine. It is rapidly metabolized to its major urinary metabolite norfentanyl through oxidative N-dealkylation at the piperidone ring primarily by the hepatic enzyme CYP3A5. Fentanyl activity/detection can vary depending on genetic variation of enzymatic activity and concomitant use of drugs that may inhibit (i.e. macrolides, antifungals, cimetidine) or induce (i.e. carbamazepine, phenytoin) CYP3A5. Fentanyl is estimated to be 50-100 times more potent than morphine and 25-40 times more than heroin. Potent fentanyl analogs have now been developed with even higher abuse potential and risks. The new "designer" opiates derived from fentanyl are now cheaper than heroin making them more accessible and of even greater concern in cases of addiction [33]. Analysis of umbilical cord tissue does avoid detection of substances administered after birth, however, may erroneously detect drugs during labor and at delivery if proper cleansing and rinsing is not properly performed to remove any residual maternal blood. The mother in this this Case did not undergo an epidural and/or receive Fentanyl prior to delivery. Fentanyl exposure in the later period of pregnancy but not recent enough to be detected in the maternal urine specimen is further supported since there was enough time elapsed to produce the metabolite norfentanyl. There again is the possibility meconium analysis did not demonstrate an earlier exposure of fentanyl/norfentanyl during pregnancy because concentrations may have been below defined assay positivity cut off levels.

5. Conclusions

We present maternal/newborn dyad toxicology reports from cases a clinician may similarly be faced to interpret, and we have attempted to provide guidance to review discordant results from the various analyzed tissue types. Proper interpretation of these tests is essential as prenatal drug testing aims to detect intrauterine drug exposure to assist in the management of withdrawal and used by social services/child protective agencies to assist in difficult decisions regarding the best care of the infant.

Neonatal drug testing has been reported in several specimen types including urine, umbilical cord, meconium, and hair [30, 31]. The selection of specimens for drug testing are based on several factors, some of which are scientific (window of detection and chemical properties) and some of which are practical (specimen availability and collection). There is no FDA approved guidance for toxicological testing of perinatal drug exposure, therefore tests from reference and commercial laboratories might be discordant because of variables in screening methodologies/confirmatory analysis, detection value cut-offs, drug panels, and sample preparation.

Both umbilical cord tissue and meconium have a place in the confirmation of *in utero* substance exposure. Those interpreting the results need to be aware that umbilical cord tissue and meconium might not produce concordant results and should understand reasons for the discordance between the two. As alluded the meconium accumulating process for intrauterine drug exposures provides a longer-term window of detection that can extend back to the second trimester of pregnancy. Many substances of abuse are lipid-soluble and are favored to accumulate in meconium. Since meconium is not normally excreted before birth, it becomes the perfect reservoir to identify drug exposures from an early stage of pregnancy. To obtain enough meconium however for testing, collection may take several days especially from those with delayed passage of stool such as seen with premature infants and infants exposed prenatally to opiates [12, 16]. The greater the delay in collection also poses an increasing risk the meconium specimen will be contaminated by medications administered to the infant after delivery, with urine, transition stool and inaccuracies if multiple collections are not completely blended [12, 16]. Prolonged specimen collection also poses the risk of drug/metabolite degradation resulting in false negative results (16). And finally, meconium may not be available for testing such as when there is in utero passage. Despite these challenges, meconium remains the gold standard for in utero drug exposure testing.

Umbilical cord tissue has become an alternative for

newborn drug screening and as demonstrated in the above cases can complement defining drug exposures. Umbilical cord is readily available after birth and of ample quantity which allows an expedited turn around for results compared to meconium analysis. Proper processing of the cord however is critical to guarantee accuracy. The cord must be drained of blood and rinsed thoroughly to prevent contamination and detection of drugs administered during labor and/or delivery. The formation of the cord is by the fifth-seventh week of gestation with much of its development occurring in the third trimester thus limiting detection of drug exposures occurring in the first/second trimester. Drug/metabolite concentrations are frequently lower in umbilical cord tissue compared to meconium with deposition occurring in the Wharton jelly [16]. Wharton jelly is a gelatinous connective tissue made up of glycosaminoglycans such as hyaluronic acid, collagen fibers, myofibroblasts and mast cells. Wharton jelly is much less hydrophobic than meconium and since most drugs of abuse are relatively hydrophobic, they will deposit in meconium at a greater concentration [35]. Drug deposition in meconium compared to Wharton jelly is also favored because repeated swallowing of amnionic fluid tends to concentrate and preserve drug/metabolite physicochemical properties, while drug/metabolite deposition in umbilical cord tissue (hydrophilic in nature, less lipophilic) may undergo fetal metabolism and affect the sensitivity of the umbilical cord matrix for detection of certain drugs [16]. Optimization of instrumentation and methods for umbilical cord tissue toxicology are still needed to achieve comparable sensitivity to meconium.

Urine drug testing has also been a mainstay to identify a possible infant drug exposure, but because of its limited window for detection for accuracy it should be used in a combination with the infant's clinical presentation and other toxicology/laboratory results. Hair is also a valid matrix for drug screening but rarely used and not tested or discussed in any of the above presented Cases. Fetal hair becomes evident during the third trimester and can provide evidence of third trimester drug exposure and is dependent on the length of hair analyzed. Limitations with drug testing neonatal hair can include variation in drug deposition based on hair type, limited availability of sufficient quantity for testing, objection to removal of hair and a sparse number of laboratories that will test hair [7]. At our facility we have not used hair for neonatal drug screening and if we did it would require parental consent.

Meconium, umbilical cord tissue, and newborn/maternal urine are used at our facility to detect possible substance abuse during pregnancy. The presence or absence, accumulation, and concentration of drugs in these specimens are not necessarily parallel as shown in the above six Cases. Many factors are involved with these discrepancies, including time of drug exposure, detection window for drugs and metabolites, chemical properties of the drugs and their metabolites, as well as pre-analytical and analytical variables.

In conclusion, the cases presented exemplify the discordancy between urine, meconium and umbilical cord tissue that are used for prenatal and newborn drug screening. Discussion of these cases serves to also provide some possible explanation for the results obtained from the various specimens tested.

Conflict of Interest

The authors declare that they have no competing interests.

Author Contribution

NJA was responsible for collection and review of data and assisting with writing of the manuscript, TWR assisted with review of information and writing of the manuscript, CNS assisted with data and SAJ assisted as an expert in the field, interpretation of testing results and review of the manuscript.

Previous Presentation

Academy of Clinical Laboratory Physicians and Scientists (ACLPS), 2020.

American Association for Clinical Chemistry (AACC), 2020.

Abbreviations

Abbreviations	Definitions
HPLC-MS/MS	High performance liquid chromatography tandem mass spectrometry
NAS	Neonatal Abstinence Syndrome
AMP	Amphetamine
MAMP	Methamphetamine
THC	Tetrahydrocannabinol
EDDP	5-Dimethyl-3, 3-diphenylpyrrolidine
CYP2D6	Cytochrome P450 2D6
EMDP	2-Ethyl-5-methyl-3,3-diphenylpyroline
CYP 450	Cytochrome P450
m-OH-BZE	meta-hydroxy-benzoylecgonin

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